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|--|--|-----------|---|
| (51) International Patent Classification ⁷ : C12N 15/00, A01K 67/027, C07K 14/81 | | A3 | (11) International Publication Number: WO 00/26357 |
| | | | (43) International Publication Date: 11 May 2000 (11.05.00) |
| (21) International Application Number: PCT/US99/25710 | | | (72) Inventors; and (75) Inventors/Applicants (for US only): ECHELARD, Yann [US/US]; 248 Moss Hill Road, Jamaica Plains, MA 02131 (US). BEHBODI, Esmail [US/US]; 45 Shrewsbury Common Drive #48, Shrewsbury, MA 01545 (US). ZIOMEK, Carol [US/US]; 17 Shadowbrook Lane #6, Milford, MA 01757 (US). GAVIN, William [US/US]; 86 Dudley Oxford Road, Dudley, MA 01571 (US). MELICAN, David [US/US]; 296 Brookfield Road, Fiskdale, MA 01518 (US). (74) Agent: MYERS, Louis; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US). (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 23 November 2000 (23.11.00) |
| (22) International Filing Date: 2 November 1999 (02.11.99) | | | |
| (30) Priority Data: 60/106,728 2 November 1998 (02.11.98) US 09/298,508 22 April 1999 (22.04.99) US 09/298,971 23 April 1999 (23.04.99) US 60/131,328 26 April 1999 (26.04.99) US | | | |
| (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications US 60/106,728 (CIP) Filed on 2 November 1998 (02.11.98) US 09/298,508 (CIP) Filed on 22 April 1999 (22.04.99) US 09/298,971 (CIP) Filed on 23 April 1999 (23.04.99) US 60/131,328 (CIP) Filed on 26 April 1999 (26.04.99) | | | |
| (71) Applicant (for all designated States except US): GENZYME TRANSGENIC CORP. [US/US]; Five Mountain Road, P.O. Box 9322, Framingham, MA 01701-9322 (US). | | | |
| (54) Title: TRANSGENIC AND CLONED MAMMALS | | | |
| (57) Abstract The invention features methods of making cloned and transgenic mammals, e.g., goats. The methods include making a somatic cell line, e.g., a transgenic somatic cell line which can be used as a donor cell, methods of producing a cloned or transgenic mammal by introducing the genome of a somatic cell into an enucleated oocyte, preferably a naturally matured oocyte which is telophase, to form a reconstructed embryo, and methods of transferring the reconstructed embryo. The invention also includes cell lines, reconstructed embryos and cloned or transgenic mammals. | | | |

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/25710

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/00 A01K67/027 C07K14/81

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K A01K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| A | WO 96 26268 A (GENZYME TRANSGENICS CORP) 29 August 1996 (1996-08-29) the whole document --- | 8, 14, 19, 24-32 |
| A | WO 97 07669 A (CAMPBELL KEITH HENRY STOCKMAN ; ROSLIN INST EDINBURGH (GB); WILMUT) 6 March 1997 (1997-03-06) page 17, line 18 -page 18, line 9; claims; example 5 --- | 1, 2, 6-12 |
| A | WILMUT I ET AL: "VIABLE OFFSPRING DERIVED FROM FETAL AND ADULT MAMMALIAN CELLS" NATURE, GB, MACMILLAN JOURNALS LTD. LONDON, vol. 385, no. 6619, 27 February 1997 (1997-02-27), pages 810-813, XP002067035 ISSN: 0028-0836 the whole document --- -/- | 1 |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

4 August 2000

Date of mailing of the international search report

10.08.00

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INTERNATIONAL SEARCH REPORT

Inter. Appl. No.
PCT/US 99/25710

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| A | WO 97 19589 A (NEXIA BIOTECH INC) 5 June 1997 (1997-06-05) page 4, line 23 -page 5, line 12 page 11, line 3 -page 12, line 5 page 16, line 12 -page 21, line 2; claim 5 --- | 1 |
| A | GOOTWINE, E. ET AL.: "Factors affecting success of embryo collection and transfer in a transgenic goat program." THERIOGENOLOGY, (1997) VOL. 48, NO. 3, PP. 485-499., XP000892953 page 487, last line --- | 1 |
| A | EBERT K M ET AL: "TRANSGENIC FARM ANIMALS: PROGRESS REPORT" THERIOGENOLOGY,US,LOS ALTOS, CA, vol. 39, no. 1, 1 January 1993 (1993-01-01), pages 121-135, XP000565804 ISSN: 0093-691X cited in the application the whole document --- | |
| P,X | BAGUISI A, ET AL.: "Production of goats by somatic cell nuclear transfer." NAT BIOTECHNOL, vol. 17, no. 5, May 1999 (1999-05), pages 456-461, XP000891364 the whole document --- | 1-34 |
| X | Meeting Info.: Annual Conference of the International Embryo Transfer Society Nice, France January 12-14, 1997 XP000892951 & LEWIS-WILLIAMS, J. ET AL.: "Birth of successfully identified transgenic goats using preimplantation stage embryos biopsied for fish." THERIOGENOLOGY, (1997) VOL. 47, NO. 1, PP. 226., --- | 35-54, 56-67 |
| A | BORDIGNON,V. & SMITH L.C.: "TELOPHASE ENUCLEATION: AN IMPROVED METHOD TO PREPARE RECIPIENT CYTOPLASTS FOR USE IN BOVINE NUCLEAR TRANSFER " MOLECULAR REPRODUCTION AND DEVELOPMENT, vol. 49, no. 1, January 1998 (1998-01), pages 29-36, XP000910821 US,NEW YORK the whole document ----- | 68 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/25710

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

As far as claims 7 to 32 and 103 to 106 are directed to a method of treatment of a non human mammalian body, the search has been carried out and based on the alleged effects of the composition.
2. ☒ Claims Nos.: 101
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

Claim 101 is missing. In case of payment of further fee for the search of this subject, for claim 102, that refers to "the method of claim 101", the search could be carried out replacing it by "the method of claim 100".
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-34

A method of producing a non human cloned mammal comprising: maintaining a mammalian reconstructed non human embryo, wherein the genome is derived from a somatic cell, in culture until the embryo is in the 2 to 8 cell stage; transferring the embryo at the 2 to 8 cell stage into a recipient mammal and allowing the reconstructed embryo to develop into a mammal, to thereby produce a non human mammal.

2. Claims: 35-67

A purified embryonic or fetal caprine somatic cell comprising a transgene wherein the cell is obtained from an embryonic or fetal goat derived from a germ cell obtained from a transgenic goat; a cell line derived from said cell; a method of preparing said cell line;

3. Claims: 68-100 102-117

A method of producing a cloned non human mammal comprising : introducing a genome from a non-human mammalian somatic cell into a naturally matured oocyte which is in telophase stage of meiotic cell division to form a reconstructed embryo and allowing the reconstructed embryo to develop into a mammal, thereby providing a non-human mammal;
a method of producing a transgenic non human mammal comprising : introducing a genome from a non-human mammalian somatic cell into a naturally matured oocyte which is in telophase stage of meiotic cell division to obtain a reconstructed embryo, and allowing the reconstructed embryo to develop into a mammal, thereby providing a non-human transgenic mammal;
a method of making a cloned non human mammal comprising: fusing a non-human mammalian somatic cell with a naturally matured enucleated non-human oocyte which is in telophase stage of meiotic cell division to form a reconstructed embryo, activating the reconstructed embryo, transferring the embryo in a doe and allowing the embryo to develop into a non-human mammal;
a method of making a transgenic non human mammal comprising : fusing a non-human mammalian somatic cell with a naturally matured enucleated oocyte which is in telophase stage of meiotic cell division to obtain a reconstructed embryo; activating the reconstructed embryo, transferring the embryo in a doe and allowing the to develop into a non human mammal; a cloned or transgenic non human mammal obtained by

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

said methods.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 101

Claim 101 is missing. In case of payment of further fee for the search of this subject, for claim 102, that refers to "the method of claim 101", the search could be carried out replacing it by "the method of claim 100".

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 99/25710

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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| WO 9626268 A | 29-08-1996 | US 5843705 A AU 695249 B AU 5026296 A CA 2197675 A EP 0773992 A | 01-12-1998 13-08-1998 11-09-1996 29-08-1996 21-05-1997 |
| WO 9707669 A | 06-03-1997 | AU 716956 B AU 6831096 A BR 9610034 A CA 2229568 A CN 1202084 A CZ 9800608 A EP 0849990 A EP 0930009 A EP 1005789 A GB 2318578 A,B GB 2331751 A,B HU 9900234 A JP 2000506722 T NO 980845 A NZ 316149 A PL 325331 A | 09-03-2000 19-03-1997 21-12-1999 06-03-1997 16-12-1998 15-07-1998 01-07-1998 21-07-1999 07-06-2000 29-04-1998 02-06-1999 28-05-1999 06-06-2000 29-04-1998 28-10-1999 20-07-1998 |
| WO 9719589 A | 05-06-1997 | US 5907080 A AU 721132 B AU 7706496 A CA 2239180 A EP 0871357 A JP 2000501287 T NZ 323016 A | 25-05-1999 22-06-2000 19-06-1997 05-06-1997 21-10-1998 08-02-2000 30-08-1999 |